The sprout inhibitor 1,4-dimethylnaphthalene alters the expression of genes in potato eyes associated with stress and cell viability

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Abstract:

The environmental and health concerns associated with the sprout control agent (CIPC) have resulted in the development of new compounds for prolonging the storage of potato tubers. One such compound, dimethylnaphthalene (DMN), was originally isolated from potato skins and is used both in association with CPIC or alone to prevent sprouting in harvested tubers. In order to elucidate the mode of action for DMN RNA-seq was used to examine gene expression changes in non-dormant potato tubers (cv. Russet Burbank) treated with increasing amounts of this growth inhibitor. Potato tubers were treated in an airtight chamber with varying concentrations of DMN to yield skin residue levels of 0.15, 1.38, 2.13, and 4.2 ppm. Potato eyes were excised, frozen in liquid nitrogen, and stored at -80C. Total RNA was isolated from frozen meristems, quantified, and used to make cDNA. Samples were sequenced using Illumina technology and were mapped to the potato genome using the Tuxedo suite. Exposure of potato meristems to 2.13 ppm of DMN had reduction of transcripts associated with cell proliferation. At higher DMN exposures a number of WRKY transcription factors and other genes associated with cell stress and possibly apoptosis were induced.

Introduction:

One of the most common compounds used to prevent premature sprouting in potato tubers during storage is chlorpropham (CIPC). CIPC functions through the disruption of mitotic spindles and prevention of cell division. There have been some concerns regarding the possible health effects of residual CIPC and the use of CIPC on tubers precludes their use as seed stock. Thus, there is interest in developing alternative compounds that can be used to control postharvest sprouting in potato tubers. The compound 1,4-dimethylnaphthalnene, originally isolated from potato tubers, has been shown to be useful as a sprout control agent with the ability to reversibly prevent sprouting in seed stock. How DMN functions as a sprout control agent is unknown. What is known is that CIPC and DMN do not function through a similar mechanism. CIPC prevents arrests cell division in the mitotic phase of the cell cycle while DMN arrests cells in the S-phase prior to DNA replication. Gene expression analysis using microarrays has shown that DMN alters gene expression in potato meristems and it may do so by increasing the expression of the cell cycle inhibits KRP1 and KRP2.

In this study we expanded on the functional analysis of DMN as a sprout control agent by conducting detailed expression studies through the use of RNA-seq. This approach enables us to examine gene expression on a global scale, map the specific gene changes to the potato genome, and begin to build a transcriptional map outlining sprout control in potato.

Methods:

Plant Material

Potato tubers were harvested in the fall of 2012 and 2013 and stored at 4 C until dormancy release. Tubers were placed in single layer at the bottom of a 9.5 Liter BBL GasPak chamber. Whatman filter paper spotted with DMN was suspended in wire racks above the tubers and the chambers were sealed and incubated at 20 C for two days. DMN amounts ranged from 0, 1, 2.5, 7.5, and 30 ul of DMN per liter of chamber air space. Following the two-day incubation chambers were opened in a fume hood. Tubers were removed to a wire basket and placed in a growth chamber overnight at 20 C. Two cm periderm plugs were then taken from each tuber and set to Dichlor Analytical Laboratory (Meridian, ID) for DMN residue analysis. An average ppm residue was determined for each treatment.

RNA-seq

Tubers meristems were excised using a microcurette, quick frozen in liquid nitrogen, and stored at -80C until RNA isolation. Total RNA was isolated by grinding meristems to a powder in a mortar and pestle followed by extraction using a Ribopure Kit (www.ambion.com). RNA was quantified using a BioSpec Nano and quality was measured using an Agilent 2100 Bioanalyzer (www.agilent.com). Samples having an RNA Integrity Number (RIN) of greater than 7.6 was used for analysis. Samples were shipped to the Nucleic Acid Core Facility at Penn State University Park for Illumina sequencing. Sequences were mapped to the double haploid potato genome (*Solanum tuberosum phureja)* using the Galaxy suite (https://usegalaxy.org) and the program Tophat. Following mapping gene expression changes between different DMN treatments were determined using Cufflinks.

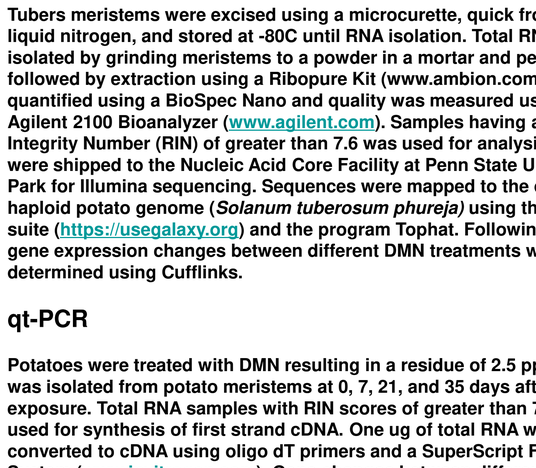
qt-PCR

Potatoes were treated with DMN resulting in a residue of 2.5 ppm. RNA was isolated from potato meristems at 0, 7, 21, and 35 days after DMN exposure. Total RNA samples with RIN scores of greater than 7.6 were used for synthesis of first strand cDNA. One ug of total RNA was converted to cDNA using oligo dT primers and a SuperScript First-Strand System (www.invitrogen.com). Gene changes between different DMN treatments were determined using a ΔΔCT method with the gene EF1-α as the internal control. EF1-α was chosen as the reference based on the RNA-seq expression data, which showed no statistical expression difference between different DMN treatments.

WRKY Gene Analysis

The *Solanum tuberosum phureja* (double haploid genome) http://solgenomics.net was searched using tBLASTx for WRKY-type transcriptions factors using known genes from the *Arabidopsis thaliana* genome. The putative peptide sequences were aligned using MAFFThttp://www.ebi.ac.uk/Tools/msa/mafft/.

Procedural outline for RNA-seq analysis of transcriptional changes associated with DMN treatment



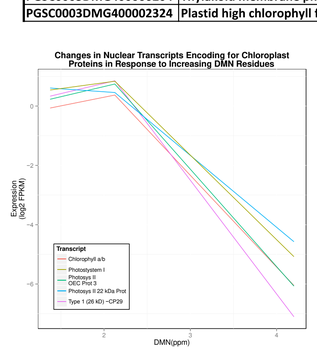
Results:

RNA-seq

- **A total of 1.49 X 10^8 RNAs were sequenced from potato meristems treated with differing DMN concentrations.**
- **35,092 unique RNAs mapped to putative coding regions within the potato genome.**
- **2142 mapped transcripts exhibited statistically significant change in expression in response to DMN**
- **Higher levels of DMN resulted in a decreases of many genes associated with chloroplast structure or photosynthesis (See below).**
- **Levels of a number of WRKY-type transcripts changed in response to DMN (See below).**

DMN and Plastids (RNA-seq Data)

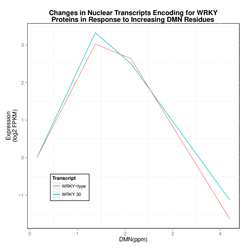
Table of Transcripts Repressed by DMN Exposure that Encode for Plastid Proteins	
Gene ID	**Putative Function of Plastid Protein**
PGSC0003DMG400006149	Chlorophyll a-b binding protein 4, chloroplastic
PGSC0003DMG400019584	Ribulose bisphosphate carboxylase small chain 1, chloroplastic
PGSC0003DMG400013460	Chlorophyll a-b binding protein 3C, chloroplastic
PGSC0003DMG400021727	Photosystem II oxygen-evolving complex protein 3
PGSC0003DMG400008297	Chlorophyll a-b binding protein 1B, chloroplastic
PGSC0003DMG400014386	Chlorophyll a-b binding protein 7, chloroplastic
PGSC0003DMG400008488	Chloroplast pigment-binding protein CP29
PGSC0003DMG400008301	Chlorophyll a/b binding protein
PGSC0003DMG400008564	Chlorophyll a-b binding protein 13, chloroplastic
PGSC0003DMG400013412	Chlorophyll a-b binding protein 3C
PGSC0003DMG400000926	Oxygen-evolving enhancer protein 2, chloroplastic
PGSC0003DMG400021287	Chlorophyll a-b binding protein 8, chloroplastic
PGSC0003DMG400010035	Oxygen-evolving enhancer protein 1, chloroplastic
PGSC0003DMG400007536	Photosystem II reaction center W protein, chloroplastic
PGSC0003DMG400021144	Photosystem I subunit III
PGSC0003DMG400027276	Mg protoporphyrin IX chelatase
PGSC0003DMG400002782	Oxygen-evolving enhancer protein 1, chloroplastic
PGSC0003DMG400022022	Photosystem I reaction center subunit IV B isoform 2
PGSC0003DMG400011816	Photosystem I reaction centre PSI-D subunit
PGSC0003DMG400019149	Ribulose bisphosphate carboxylase/oxygenase activase, chloroplastic
PGSC0003DMG400015356	NADPH:protochlorophyllide oxidoreductase
PGSC0003DMG400022241	Photosystem II 10 kDa polypeptide, chloroplastic
PGSC0003DMG400016482	ATP synthase gamma chain, chloroplastic
PGSC0003DMG400042093	Chloroplast photosystem II subunit X
PGSC0003DMG400025007	NADPH:protochlorophyllide oxidoreductase
PGSC0003DMG400012494	PGR5 1A, chloroplastic
PGSC0003DMG402000895	Chlorophyll synthase
PGSC0003DMG400027013	Tetrapyrrole-binding protein, chloroplast
PGSC0003DMG400012591	Chlorophyll a-b binding protein CP24 10A, chloroplastic
PGSC0003DMG400012590	Chlorophyll a-b binding protein CP24 10B, chloroplastic
PGSC0003DMG400013751	Cytochrome b6-f complex iron-sulfur subunit, chloroplastic
PGSC0003DMG400009956	CDSP32 protein (Chloroplast Drought-induced Stress Protein of 32kDa)
PGSC0003DMG400022088	Transketolase, chloroplastic
PGSC0003DMG400002626	Photosystem I psaH protein
PGSC0003DMG402028574	Thylakoid lumen 18.3 kDa protein
PGSC0003DMG400006208	Plastoquinol-plastocyanin reductase
PGSC0003DMG400018351	NADPH:protochlorophyllide oxidoreductase
PGSC0003DMG400013027	Acetolactate synthase 1, chloroplastic
PGSC0003DMG400000204	Thylakoid membrane phosphoprotein 14 kDa, chloroplast
PGSC0003DMG400002324	Plastid high chlorophyll fluorescence 136



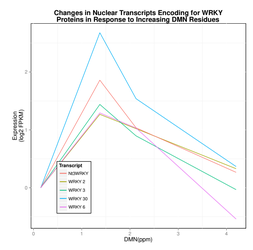
Low levels of DMN have little effect on the expression of transcripts encoding for plastid proteins. DMN levels above 2ppm result in a decrease of many plastid associated transcripts. It should be noted that RNA was isolated and sequenced from excised meristems, which are not photosynthetically active. Repression of plastid proteins is not restricted to the process photosynthesis alone but also to proteins associated with plastid structure and chlorophyll biosynthesis. We hypothesize that DMN levels above 2ppm repress both plastid development as well as photosynthesis.

DMN and WRKY Transcription Factors (RNA-seq Data)

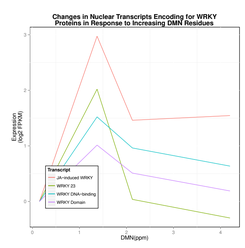
WRKY-type Transcription Fators that Change in Response to DMN	
Gene ID	**Function**
PGSC0003DMG400000064	WRKY transcription factor 23
PGSC0003DMG400000211	WRKY transcription factor
PGSC0003DMG400005835	WRKY transcription factor-30
PGSC0003DMG400007947	WRKY transcription factor 2
PGSC0003DMG400009530	WRKY transcription factor 3
PGSC0003DMG400011633	WRKY-type transcription factor
PGSC0003DMG400012160	WRKY transcription factor-30
PGSC0003DMG400016441	WRKY protein
PGSC0003DMG400016769	Double WRKY type transfactor
PGSC0003DMG400019824	JA-induced WRKY protein
PGSC0003DMG400021895	WRKY-type DNA binding protein
PGSC0003DMG400024961	WRKY domain class transcription factor
PGSC0003DMG400028520	WRKY transcription factor 1
PGSC0003DMG400029207	WRKY transcription factor 6
PGSC0003DMG400029371	DNA-binding protein NtWRKY3



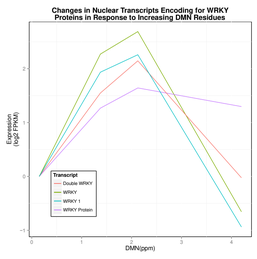
WRKY transcripts induced by DMN with a maximum expression at 1.38 ppm and a repression of expression at 4.2 ppm.



WRKY transcripts induced by DMN with a maximum expression at 1.38 ppm and a decrease in expression at higher DMN levels.

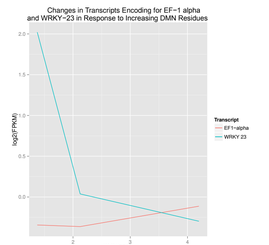


WRKY transcripts induced by DMN with a maximum expression at 1.38 ppm and a relatively level expression at higher DMN exposures.

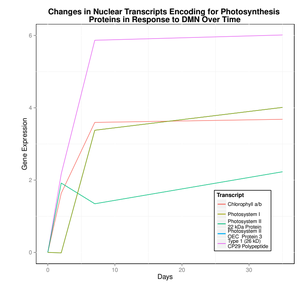


WRKY transcripts induced by DMN with a maximum expression at 2.13 ppm.

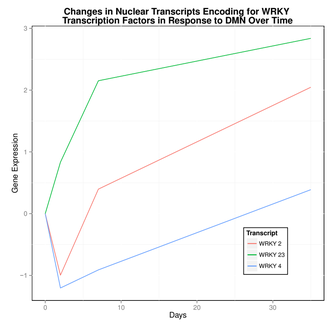
qtPCR analysis of gene expression



EF1-alpha was chosen as a reference gene based on RNA-seq experiments, which indicated that EF1-alpha expression does not change significantly as DMN exposure increases.

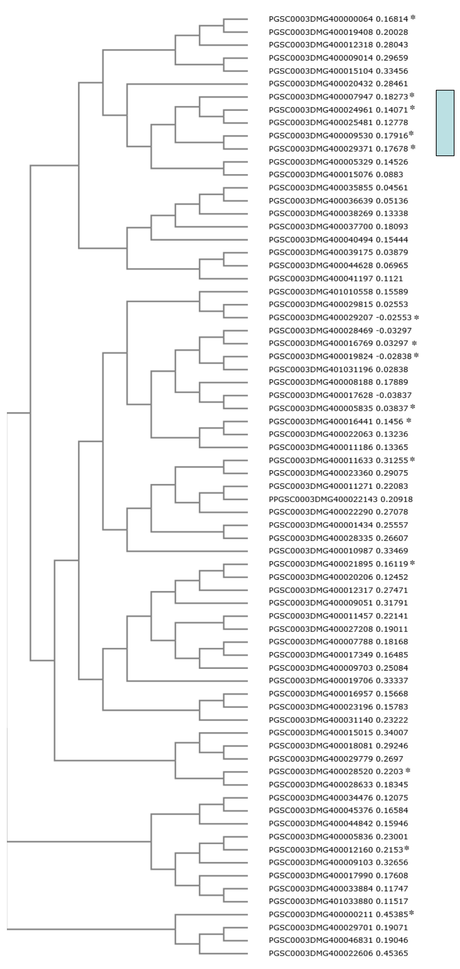


qtPCR analysis of nuclear encoded plastid transcripts. All samples were treated with DMN resulting in an average residue of 2.5 ppm. Expression of transcripts was determined at 0, 2,7, 21, and 35 days following DMN exposure.



qtPCR analysis of a subset of the WRKY-type transcripts. All samples were treated with DMN resulting in an average residue of 2.5 ppm. Expression of transcripts was determined at 0, 2,7, 21, and 35 days following DMN exposure.

WRKY-type transcription factors found in the potato genome



MAFFT alignment of putative amino acid sequences for WRKY-type transcription factors found in the potato double haploid genome (*Solanum tuberosum phureja).* Accessions marked with * have been found to change in expression after exposure of potato tubers to DMN. The blue box represents a related group of WRKY transcripts that respond to DMN.

CONCLUSIONS:

1. DMN exposure reduces the expression of transcripts associated with plastid development and photosynthesis.
   a. Expression of plastid related transcripts began to return to pre-DMN exposure levels after ten days.
2. DMN exposure alters the expression of some WRKY-type transcription factors.
   a. The WRKY-transcription factors fall into subgroups that exhibit similar responses to DMN in a dose dependent manor.

ACKNOWLEDGEMENTS:

Portions of this work were funded by a grant from the 1,4-Group of Meridian, ID.

In dedication to Jim Zalewsky